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PRC2 represses dedifferentiation of mature somatic cells in Arabidopsis

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Plant somatic cells are generally acknowledged to retain totipotency, the potential to develop into any cell type within an organism. This astonishing plasticity may contribute to a high regenerative capacity upon severe damages but how plants control this potential during normal post-embryonic development remains largely unknown^{1, 2}. Here we show that POLYCOMB REPRESSIVE COMPLEX 2 (PRC2), a chromatin regulator that maintains gene repression through histone modification, prevents dedifferentiation of mature somatic cells in *Arabidopsis thaliana* roots. Loss-of-function mutants in PRC2 subunits initially develop unicellular root hairs indistinguishable from those in wild-type but fail to retain the differentiated state, ultimately resulting in the generation of an unorganised cell mass and somatic embryos from a single root hair. Strikingly, mutant root hairs complete the normal endoreduplication programme, increasing their nuclear ploidy, but subsequently reinitiate mitotic division coupled with successive DNA replication. Our data show that the *WOUND INDUCED DEDIFFERENTIATION3* (*WIND3*) and *LEAFY COTYLEDON2* (*LEC2*) genes are among the PRC2 targets involved in this reprogramming as their ectopic overexpression partly phenocopies the dedifferentiation phenotype of PRC2 mutants. These findings unveil the pivotal role of PRC2-mediated gene repression in preventing unscheduled reprogramming of fully differentiated plant cells.

The remarkable developmental plasticity of plant cells was already documented in a pioneering study in the 1950s that demonstrated the regeneration of whole plant bodies from fully differentiated somatic cells *in vitro*³. Plant cells exert this potential upon severe damages but intact plants hardly develop callus, unorganised cell mass, or somatic embryos under favourable conditions¹. Plants likely possess mechanisms to prevent unscheduled dedifferentiation but little is known on this control. To identify regulatory mechanisms that suppress reprogramming of differentiated cells, we collected a set of *Arabidopsis* mutants that display callus phenotypes¹ and subscreened for those that initiate ectopic proliferation of mature cells. We reasoned that root hairs represent a suitable system for this study since they have a unicellular structure on the root

epidermis with highly specialised functions in water and nutrient uptake⁴. As shown in Fig. 1a, we found that the concomitant loss of CURLY LEAF (CLF) and SWINGER (SWN), plant homologs of the *Drosophila* E(z) subunit in PRC2⁵, leads to the development of multicellular root hairs. At 15 days after germination, 96 % of *clf-28 swn-7* plants (n = 23) display multicellular root hairs that are never observed in wild-type (WT) plants. Mutations in other PRC2 subunits, such as the Esc homolog, FERTILIZATION INDEPENDENT ENDOSPERM (FIE)⁶, and the Su(z)12 homologs, EMBRYONIC FLOWER2 (EMF2) and VERNALIZATION2 (VRN2)⁷, also cause extensive division of root hairs at a similar frequency (Fig. 1), pointing to the requirement of PRC2 activity in suppressing ectopic proliferation of differentiated cells. Remarkably, sustained divisions ultimately lead to the formation of calli, some of which further develop into somatic embryos that show typical accumulation of lipids (Fig. 1a).

Several lines of evidence demonstrate that PRC2 deficiency leads to mitotic reactivation in terminally differentiated root hairs. First, the root hair-specific differentiation marker *pEXP7:NLS-GFP*⁸ shows a similar expression pattern in WT, heterozygous and homozygous *emf2-3 vrn2-1* roots, indicating that in the absence of PRC2 root hair differentiation occurs indistinguishably from WT (Fig. 1b). In addition, root hairs of 7-day-old *emf2-3 vrn2-1* plants are initially unicellular and only become multicellular in older plants (Fig. 1c). Serial observations of *emf2-3 vrn2-1* roots expressing plasma membrane (LTI6-GFP) and nucleus (H2B-YFP) markers⁹ further indicate that only fully elongated root hairs undergo nuclear and cellular division (Fig. 1d). Multicellularisation of root hairs does not appear to follow a typical gradient along the root axis, suggesting that it is not correlated with the timing of their initial differentiation (Supplementary Fig. 1). Time-lapse imaging of *emf2-3 vrn2-1* root hairs expressing LTI6-GFP and H2B-YFP confirms that these nuclear divisions are accompanied by the formation of a new cell plate and are therefore clearly distinct from the nuclear fragmentation occasionally observed in WT root hairs¹⁰ (Fig. 1e, Supplementary Video 1).

As part of the differentiation program, Arabidopsis root hairs undergo several rounds of

endoreduplication, a modified cell cycle in which cells replicate nuclear DNA without mitoses and concomitantly increase nucleus and cell size¹¹. Entry into the endoreduplication cycle is generally accepted as a commitment for terminal differentiation since cells that have endoreduplicated do not normally reenter the mitotic cycle¹². Flow cytometry analysis showed that activity of the *EXP7* promoter, the pattern of which is similar between WT and PRC2 mutants, is almost exclusively associated with 8C and 16C nuclei (Supplementary Fig. 2), suggesting that root hairs in PRC2 mutants undergo endoreduplication before becoming multicellular. Correspondingly, as previously reported for *fie* seedlings⁶, cells in *clf-28 swi-7*, *fie* and *emf2-3 vrn2-1* roots endoreduplicate and increase ploidy levels up to 16C, which is similar to that observed for WT (Supplementary Fig. 3a). Furthermore, nuclear size, measured on DAPI-stained or H2B-YFP-labelled nuclei, is comparable between unicellular, fully elongated root hairs in WT and PRC2 mutants (Supplementary Fig. 3b, d). Quantitative measurements of SYBR GREEN-stained nuclei also show that chromocentre size, which correlates with ploidy level¹³, is similar between WT and unicellular root hairs in PRC2 mutants (Fig. 1f, Supplementary Fig. 3c), indicating that they have similar ploidy levels. Consistent with these observations, the mitotic B-type cyclin, CYCB1;2, which is normally present in proliferating meristem cells but not in differentiated root hairs¹⁴, is ectopically expressed in the dividing hairs of *clf-28 swi-7* roots (Fig. 1g, Supplementary Video 2). Importantly, other cell types, such as cortex cells that also endoreduplicate, display similar ectopic nuclear division in *clf-28 swi-7* (Supplementary Fig. 4, Supplementary Video 3), suggesting that PRC2 is generally required to prevent mitotic re-entry in mature root cells.

We next investigated whether the division of endoreduplicated hair nuclei is accompanied by DNA replication or whether they undergo reductive division without DNA replication. Two lines of evidence indicate that PRC2 deficiency leads to mitotic division coupled with DNA replication. First, nuclear size of multicellular root hairs in *emf2-3 vrn2-1* is similar to that of endoreplicated root hairs in WT (Supplementary Fig. 2e), suggesting that the high ploidy level is maintained in the mutant. Second, fluorescence microscopy analysis revealed that 5-ethynyl-2'-deoxyuridine (EdU, a

thymidine analog) is incorporated into the DNA of endoreplicated nuclei of *emf2-3 vrn2-1* hair cells (Fig. 1h) but not in WT. Taken together, these results demonstrate that single, terminally differentiated root cells possess the potential to dedifferentiate and that PRC2 suppresses this capacity *in vivo*.

Consistent with our finding that PRC2 is required to maintain the differentiation status, PRC2 components are expressed in differentiating root cells (Supplementary Fig. 5). PRC2 activity maintains gene repression by catalysing the trimethylation of histone H3 at lysine 27 (H3K27me3), which is almost entirely lost in *clf swm*, *emf2 vrn2* and *flc*^{6, 15, 16}. Several embryonic or meristem regulators induce cell proliferation when overexpressed, often leading to callus formation and/or somatic embryogenesis¹. Given that many of these regulators are targeted by PRC2¹⁷, derepression of these genes might underlie cellular dedifferentiation observed in PRC2 mutants. Quantitative RT-PCR analysis indeed revealed that among numerous embryonic or meristem regulators marked by H3K27me3 in WT roots^{18, 19}, the expression of *LEAFY COTYLEDON1 (LEC1)*, *LEC2*, *FUSCA3 (FUS3)*, *WUSCHEL (WUS)*, and *WUSCHEL-RELATED HOMEODOMAIN5 (WOX5)* is strongly elevated in *emf2-3 vrn2-1* roots (Fig. 2). Other PRC2-targeted loci, such as *AGAMOUS-LIKE15 (AGL15)*, *BABY BOOM (BBM)*, *PLETHORA1 (PLT1)*, and *PLT2* did not show marked changes in expression level. *WOUND INDUCED DEDIFFERENTIATION (WIND)* genes represent another class of key regulators that promote callus generation upon overexpression²⁰. Accordingly, the expression of *WIND1*, *WIND2* and *WIND3* is upregulated in *emf2-3 vrn2-1* and *WIND1* accumulation tightly correlates with root hair dedifferentiation (Fig. 2, Supplementary Fig. 6). Analyses of epigenomic data from different tissues including hair cells^{19, 21} revealed that only *WIND3* is marked by H3K27me3 in all tissues examined while all four *WIND* genes are marked by H3K4me3, a chromatin modification associated with active transcription (Fig. 3a, Supplementary Fig. 7). Our chromatin immunoprecipitation (ChIP) experiments coupled with quantitative real-time PCR validated the preferential enrichment of H3K27me3 at the *WIND3* locus in 14-day-old WT roots (Fig. 3b), confirming that, among *WIND* genes, only *WIND3* is directly targeted by PRC2 at

this developmental stage.

We next investigated whether ectopic overexpression of any of these PRC2 target genes causes multicellular root hairs. As shown in Fig. 4a, *35S:WIND3* plants develop unicellular root hairs similar to those in WT after germination that progressively turn multicellular and develop callus. *35S:WIND1* and *35S:WIND2* plants also develop multicellular root hairs (Fig. 4a), indicating that they also trigger root hair dedifferentiation. Overexpression of *LEC2* using *35S:LEC2-GR*²² induces root hair multicellularisation (Fig. 4a) and concomitant activation of *WIND1*- and *LEC2*-mediated pathways enhances this phenotype (Supplementary Fig. 8), suggesting that PRC2 plays an integrative role in repressing multiple developmental pathways. Introducing the *lec2* mutation partially suppresses the multicellular phenotype in *clf-28 swm-7* since the proportion of multicellular root hairs reduces from 56% in *clf-28 swm-7* (n = 71) to 17% in *clf-28 swm-7 lec2* (n = 81) (Fig. 4b), indicating that ectopic *LEC2* expression underlies root hair multicellularisation. We did not observe the same suppression phenotype when introducing the *wind3* mutation in *clf-28 swm-7*, suggesting higher levels of redundancy in the WIND3-dependent pathway.

This study reveals that PRC2 activity is required to prevent unscheduled dedifferentiation of terminally differentiated plant cells (Fig. 4c). This is an important discovery since **what mechanism limits cellular plasticity in plant development and whether such a mechanism is even necessary have remained unsolved for many decades^{1,2}**. Several recent studies showed that hormone-induced callus in Arabidopsis tissue culture derives from adult stem cells in the pericycle, **indicating that callus formation does not necessarily involve dedifferentiation of mature somatic cells^{23, 24, 25}**. Previous studies reporting callus formation or somatic embryogenesis in PRC2 mutants^{5, 6} or **WIND/LEC2 overexpressors^{1, 20, 26}** did not explicitly show dedifferentiation of mature somatic cells **but our data in this study clearly demonstrate that even fully differentiated, endoreduplicated cells can still dedifferentiate and produce somatic embryos once PRC2-mediated epigenetic repression is removed**. Why PRC2 deficiency does not cause dedifferentiation earlier in development remains an open question **but we postulate that this might be linked to the fact that the main function of PRC2**

is to maintain transcriptional decisions over time rather than taking these decisions. In fact, the apparent delay in observing developmental defects is a common feature of phenotypic alterations associated with PRC2 deficiency^{6, 27}. It is plausible that during early development genetically defined transcriptional networks sustain proper growth and differentiation until their robustness becomes sufficiently challenged, for example, by the ectopic expression of PRC2-targeted reprogramming regulators such as *WIND3* and *LEC2*. In nature various forms of stresses override developmental networks to trigger cellular reprogramming¹. Uncovering how stress responses link to chromatin dynamics at key reprogramming regulators will be of great interest in future studies.

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Author Contributions

AI and KS conceived the project. MI, AI and KS designed the experiments, and MI, AI and MO conducted most of genetic and cell biological analyses except ChIP-chip which was performed by FR, AKM and JG and ploidy analyses which was performed by LDV, HH, BR and MS. CB generated *pEXP7:NLS-GFP* and *pEXP7:GTL1-GFP* plants, and MdL and SMB generated *pEMF2:EMF2-GFP* plants. MI and KS wrote the manuscript with help from co-authors.

Author Information

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Figure Legends

Figure 1 PRC2 represses dedifferentiation of mature root hair cells. **a**, Roots of WT (top left) and *clf-28 swm-7* (top right). Root hair-derived callus and embryos in *fie* (bottom). Accumulation of chlorophylls and Sudan red 7B-stained lipids highlights developing somatic embryos. Asterisks mark the main root. **b**, Root hairs of heterozygous *emf2-3/+ vrn2-1* (WT-like) and homozygous *emf2-3 vrn2-1* expressing the root hair-specific marker *pEXP7:NLS-GFP*. **c**, Root hairs of WT and *emf2-3 vrn2-1* (three lower panels) expressing the LTI6-GFP and H2B-YFP markers. **d**, Cellular and nuclear division in *emf2-3 vrn2-1* root hairs visualised by time-lapse imaging of LTI6-GFP and H2B-YFP. Colored arrows highlight nuclei originating from the same single-celled root hair. **e**, Nuclear division in *emf2-3 vrn2-1* root hair visualised by H2B-YFP. The white arrow indicates

newly formed cell plate highlighted by LTI6-GFP. **f**, SYBR GREEN-stained nuclei in WT root cap cells and WT, *clf-28 swm-7*, *emf2-3 vrn2-1* and *fie* root hairs. Note that root cap cells have 2C nuclei. **g**, Expression of the mitotic cyclin reporter CYCB1;2-YFP in *clf-28 swm-7* root tip (top) and a dividing root hair (three lower panels). **h**, EdU incorporation into nuclei of an *emf2-3 vrn2-1* root hair. Bars = 100 μ m (**a, b, c, d, g, h**), 10 μ m (**e, f**).

Figure 2 Key developmental regulators are ectopically activated in PRC2 mutant roots.

Quantitative RT-PCR analysis measuring transcript levels of embryonic, meristem and callus regulators in primary roots of 15-day-old heterozygous (WT-like) and homozygous *emf2-3 vrn2-1* plants. Transcript levels are normalised against *PP2AA3* expression and shown as fold change relative to values measured for WT-like plants (arbitrarily set to 1). Error bars represent S.E. of biological triplicates.

Figure 3 PRC2 directly targets WIND3. a, ChIP-chip analysis measuring H3K27me3 enrichment in WT seedlings, roots, root tips, epidermal root hair and non-hair cells. Green bars highlight probes reporting significant enrichment in H3K27me3 (\log_2 IP/INPUT). Red arrows show the position and orientation of *WIND* genes according to TAIR10 annotation and blue bars indicate the position of primer sets used in **b**. **b**, ChIP-qPCR validation of H3K27me3 marking in WT roots. Values represent enrichment relative to the corresponding input DNA and error bars show S.E. from three independent experiments.

Figure 4 Overexpression of WIND1, WIND2, WIND3 or LEC2 partly phenocopies the multicellular root hair phenotype of PRC2 mutants. a, Root hair phenotypes of WT, *WIND*- or *LEC2*- overexpressing plants. For WT, *35S:WIND3*, *35S:WIND1*, *35S:WIND2*, top and bottom panels show bright-field and DAPI-stained nuclei images, respectively. For *35S:LEC2-GR* grown in the presence of 10 μ M dexamethasone (DEX), top and bottom panels show bright-field and

H2B-YFP-labelled nuclei images, respectively. **b**, Partial suppression of *clf-28 swn-7* root hair phenotypes by the *lec2* mutation. Arrows mark DAPI-stained nuclei in *clf-28 swn-7* and *clf-28 swn-7 lec2* root hairs. **c**, Model for PRC2-mediated maintenance of the cellular differentiation status. PRC2 represses at least two developmental pathways (marked by black lines), one involving *WIND3* and the other *LEC2*, to prevent unscheduled dedifferentiation of mature root cells. PRC2 directly or indirectly suppresses other developmental pathways (marked by dotted lines) including those governed by *WIND1* and *WIND2*. Ectopic activation of these pathways leads to cellular dedifferentiation with subsequent callus formation and somatic embryogenesis. Bars = 0.1 mm (**a**, **b**).

METHODS

Plant materials. For phenotypic analyses, Arabidopsis PRC2 mutants, *clf-28* (At2g23380, SALK_139371) *swn-7* (At4g02020, SALK_109121)⁵ and *emf2-3* (At5g51230) *vrn2-1* (At4g16845)⁷ were crossed with *CYCBI;2-YFP*¹⁴ and *35S:LTI6-GFP 35S:H2B-YFP*⁹ plants, respectively. The embryo lethal phenotype of *fie* (At3g20740, SALK_042962) was rescued by the *cdka;1* (At3g48750, SALK_106809) mutation as described previously⁶. *35S:WIND1* (At1g78080), *35S:WIND2* (At1g22190), *35S:WIND3* (At1g36060), *wind3* (SALK_091212), *XVE:WIND1* and *pWIND1:WIND1-GFP*²⁰, *35S:LEC2-GR* (At1g28300) and *lec2* (SALK_015228)²², *pSWN:SWN-GFP*²⁸ and *pVRN2:VRN2-GUS*²⁹ were previously described. *XVE:WIND1* plants were crossed with *35S:LEC2-GR* to generate the double transgenic lines. All mutants and transgenic plants used in this study are in Columbia-0 (Col-0) background except *vrn2-1* and *pVRN2:VRN2-GUS*, which are in Landsberg erecta (Ler). Plants were grown on 0.6% (w/v) gelzan plates containing Murashige-Skoog (MS) salts and 1% sucrose under continuous light at 22°C.

Generation of transgenic plants. To generate the *pEMF2:EMF2-GFP* reporter fusion, the *EMF2* promoter was PCR amplified from genomic DNA using primers pEMF2_F (TAGAGAGAAACCCTTGTGGT) and pEMF2_R (TCTCGCTACGAGATCCTAGA). The coding sequence of *EMF2* was PCR amplified from cDNA using primers EMF2_F (CACCATGCCAGGCATTCCTCTTGTTAGTC) and EMF2_R (no stop) (AATTTGGAGCTGTTCGAGAAAG). PCR products were purified and cloned into pENTR D-TOPO (invitrogen). The *pEMF2* in pENTR 5'TA TOPO, *EMF2 cDNA* in pENTR D-TOPO and *mGFP5* in pDONR P2R-P3 were used as templates for a multisite gateway reaction with the destination vector pB7m34GW.0³⁰. The *pEXP7:NLS-GFP* plasmid was generated by PCR amplification of a 1413 bp fragment of the *EXP7* promoter using primers *pEXP7_F* (GGGGACAACCTTTGTATAGAAAAGTTGCCGTAGTTAGATGATTACAAAGGGG) and *pEXP7_R*

(GGGACTGCTTTTTTGTACAACTTGCTCTAGCCTCTTTTCTTTATTCTTAGGG) and the BAC clone T12C24 as a template. The PCR product was purified and cloned into pDONR P4P1-R (Invitrogen). The NLS fragment was obtained by annealing of the primers *NLS_F* (CACCATGGCTCCAAAGAAGAAGAGAAAGGTAGGGGGGGG) and *NLS_R* (CCCCCCCCCTACCTTTCTCTTCTTTGGAGCCATGGTG). The annealed product was cloned into pENTR-D-TOPO. The pDONR-pEXP7 and pENTR-NLS plasmids were combined by multisite gateway reaction with the destination vector R4pGWB504³¹. To obtain the *pEXP7:GTL1-GFP* fusion construct the pDONR-pEXP7, pENTR-GTL1-GFP³² and the R4pGWB501 destination vector were used in a multisite gateway reaction. All binary plasmids were introduced into the *Agrobacterium tumefaciens* strain GV3101 by electroporation and the *Agrobacterium* were infiltrated into Arabidopsis plants by the floral dip method³³.

Microscopy. For live cell imaging, plants grown on MS media were transferred onto glass bottom dishes in a high-humidity chamber to prevent the drying of root hairs. To quantify the size of nuclei and chromocentres, nuclei were stained with 4', 6-diamidino-2-phenylindole (DAPI) (Partec) and SYBR GREEN (Lonza) as previously described^{34, 35} and imaged using a BX51 fluorescence microscope (Olympus) and an SP5 confocal laser scanning microscope (Leica), respectively. The projected area of DAPI-stained nuclei and SYBR GREEN-stained chromocentres was measured using ImageJ (v. 1.49m; <http://imagej.nih.gov/ij>). To estimate the size of H2B-YFP-labelled nuclei, *emf2-3 vrn2-1* mutants harbouring *35S:H2B-YFP* were imaged using SP5 and the projected area was measured using the associated software (Leica). For the detection of DNA replication, 1 μ M of EdU (Invitrogen) was incorporated into root cells for 4 h, coupled with Alexa flour 488-labelled azide by Click-iT reaction, and imaged using BX51. Somatic embryos were stained following the protocol⁶, except that Sudan III was replaced with Sudan red 7B, and imaged using a M165FC dissection microscope (Leica).

Flow cytometry analysis. Root tips of pEXP7:GTL1-GFP plants were cut with a razor blade in 200 μ L of nuclei extraction buffer containing 45 mM $MgCl_2$, 30 mM sodium citrate, and 20 mM 3-morpholinopropane-1-sulfonic acid, pH 7.0 for 2 min, and filtered through a 50 μ m nylon filter. The DNA was stained with 1 mg/ml DAPI. Nuclei were measured using a CyFlow Flow Cytometer (Partec) excited by illumination at 375 nm and 488 nm to excite and detect DAPI (total nuclei count) and GFP-and fluorescence, respectively. The measured DNA contents were analyzed using FloMax software (Partec).

Quantitative real-time RT-PCR. Total RNA was extracted from primary roots of 15-day-old seedlings. Seeds obtained from *emf2-3^{+/-} vrn2-1^{-/-}* were sown and wild type-like siblings (*emf2-3^{+/+} vrn2-1^{-/-}* or *emf2-3^{+/-} vrn2-1^{-/-}*) and double mutants (*emf2-3^{-/-} vrn2-1^{-/-}*) were harvested for expression analyses. To minimize the contamination of dividing cells, both root apical meristems and visible lateral root primordia were removed from primary roots. Total RNA of 400 ng, isolated with RNeasy (Qiagen), was subjected for the first strand cDNA synthesis with primescript RT reagent kit (Takara). For quantitative real-time PCR, the Thunderbird SYBR qPCR mix (Toyobo) was used with the following primer sets: WIND1-F (5'-GATCTCACATCGGAGGCGATT-3'), WIND1-R (5'-CCACCGATCGAAACCGAATTC-3') for *WIND1*, WIND2-F (5'-GAGCTGACGTTTGGTGATACG-3') and WIND2-R (5'-TTACAAGACTCGAACACTGAAG-3') for *WIND2*, WIND3-F (5'-CTTCAGCTGAGCAGCCGTCAG-3') and WIND3-R (5'-ATGGCATCTCTGGCTCTTGGCA-3') for *WIND3*, WIND4-F (5'-ATCTTCCACTGCCTCAGATCG-3') and WIND4-R (5'-GTGCAAACCCATCAAGAACTC-3') for *WIND4*, WUS-F (5'-AGCCGATCAGATCCAGAAGA-3') and WUS-R (5'-AACCGAGTTGGGTGATGAAG-3') for *WUS* (At2g17950), WOX5-F (5'-GGCTAGGGAGAGGCAGAAAC-3') and WOX5-R (5'-TCCACCTTGAGTTGGAGTC-3') for *WOX5*, PLT1-F

(5'-GGTACGACGTGAAAGCCATT-3') and PLT1-R (5'-CCACCACCGTACTGGAAACT-3') for *PLT1* (At3g20840), PLT2-F (5'-CGCCTCACATTCACTCTTCA-3') and PLT2-R (5'-CGTTGGTTTGATGAATGTCG-3') for *PLT2* (At1g51190), LEC1-F (5'-CCCTTCTCTCACTATCAGCTA-3') and LEC1-R (5'-GGCATGTATTGGTCTTGCTC-3') for *LEC1* (At1g21970), LEC2-F (5'-CGAGGACGAAAGCAAGAATC-3') and LEC2-R (5'-TGCTGAAGGTCAGTGGTGAG-3') for *LEC2*, FUS3-F (5'-TGAATGCAAGGAAGGGATTC-3') and FUS3 -R (5'-CACCTAGCTGCAGACCATGA-3') for *FUS3* (At3g26790), AGL15-F (5'-CGCTCTCATAAACCACGACA-3') and AGL15-R (5'-GCTTCAGGTGGAGAATTTGC-3') for *AGL15* (At5g13790), BBM-F1 (5'-CCGTACGGATGTTGACTCCT-3') and BBM-R1 (5'-CCCAATCTCGGGAGTGACTA-3') for *BBM* (At5g17430) and PP2AA3-F (5'-GACCAAGTGAACCAGGTTATTGG-3') and PP2AA3-R (5'-TACTCTCCAGTGCCTGTCTTCA-3') for an internal control *PP2AA3* (At1g13320).

Chromatin immunoprecipitation. Whole-genome ChIP-chip data from seedlings and roots were retrieved from previous reports^{19,21}. Raw data were normalised using an ANOVA model, averaged over biological replicates, and analysed using a three-states Parsimonious Higher-Order Hidden Markov Model³⁶ as previously described³⁷. ChIP-qPCR validation of whole root samples was performed as described³⁸. Whole roots from 14 day-old WT seedlings were harvested and fixed in 1% formaldehyde under vacuum for 10 min. Fixation was stopped by the application of glycine (125 mM final concentration) and incubation under vacuum for 5 min. After the wash with distilled water, fixed tissues were frozen in liquid nitrogen, ground, re-suspended in 1 mL NIB buffer (Sigma) supplemented with DTT (1 mM final concentration) and filtered with miracloth (Calbiochem). Nuclei collected by centrifugation were subjected to 500 µL NIBA buffer (NIB buffer supplemented with 1x proteinase inhibitor cocktail, Sigma) containing 0.3% Triton-X 100 to breakdown nuclear envelope, then washed with NIBA. Chromatin was precipitated by

centrifugation and resuspended in Lysis buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% deoxychoate, 0.1% SDS, 1x plant proteinase inhibitor cocktail), then sheared by sonication to 500-1000 bp fragments using a Bioruptor (diagenode). The protein A-coated dynabeads (Life technologies) were added to the sonicated chromatin solution and the supernatant was collected using a magnetic stand after incubation for 4 h at 4°C. Then 1 µL of the antibody against H3K27me3 (#07-449, Millipore) was added to the supernatant and incubated at 4°C overnight on a rotator. After a subsequent incubation with 30 µL dynabeads for 4h at 4°C, the immune complexes were collected and washed with Lysis buffer without proteinase inhibitor, LNDET (0.25 M LiCl, 1% NP40, 1% deoxycholate, 1 mM EDTA) and TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA). The chromatin samples were then collected in elution buffer (1% SDS, 0.1 M NaHCO₃, 0.2 mg/mL proteinase K, 1 mM DTT), and incubated at 65°C overnight. RNA was subsequently degraded by incubating with RNase A (1 mg/mL) for 30 min at room temperature. After the application of 5 times volume of NTB buffer (Takara) and 1 time volume of isopropanol, DNA was purified using a MinElute PCR purification mini kit (QIAGEN) and eluted in 60 µL of EB buffer. Then 1 µL of the isolated DNA was subjected to quantitative real-time PCR using thunderbird SYBR qPCR mix (Toyobo) with the following primer sets:

(5'-CGCGTCATTCACTCATTAGC-3')		and	WIND1-1-R
(5'-GATCAGCATATTTCTATTCCAGA-3')	for	<i>WIND1-1</i> ,	WIND1-2-F
(5'-CAAATCTACTCGCTTAGGTA-3')		and	WIND1-2-R
(5'-TCTCAACCTCACCGATGAAC-3')	for	<i>WIND1-2</i> ,	WIND1-3-F
(5'-GAGAATTTCGGTTTCGATCGGT-3')		and	WIND1-3-R
(5'-CCATTGCAAGCGGCTGAATTTCC-3')	for	<i>WIND1-3</i> ,	WIND2-1-F
(5'-TCTAACCCTCTAGACCCTTC-3')	and	WIND2-1-R	(5'-
GTTCCGAATGTGACACACGT-3')	for	<i>WIND2-1</i> ,	WIND2-2-F
(5'-GGAGTACGTAAACATGGATG-3')		and	WIND2-2-R
(5'-AATAACTCTCAGCCTCACCG-3')	for	<i>WIND2-2</i> ,	WIND2-3-F

(5'-GCTGTTAACGGAGAGTTATGG-3') and WIND2-3-R (5'-ACAAGACTCGAACACTGAAG-3') for WIND2-3, WIND3-1-F (5'-CGCGTTGGCTTATGATCGC-3') WIND3-1-R (5'-TCGGCTCAGCTAATATGGCT-3') for WIND3-1, WIND3-2-F (5'-TGAGGTTGAGAGCATCTTCC-3') and WIND3-2-R (5'-GGTTTGAAGATGAAACGAAGC-3') for WIND3-2, WIND3-3-F (5'-GACCTGGTTTCTTTGGC-3') and WIND3-3-R (5'-GCGTAAGCATAAGTACAACGGT-3') for WIND3-3, WIND4-1-F (5'-GCTATAGGGAAATAGAGTTG-3') and WIND4-1-R (5'-CGCGTTGACAATTGTTATGT-3') for WIND4-1, WIND4-2-F (5'-GGTTAACTATACCGAGGCG-3') and WIND4-2-R (5'-CTGGGAAATTGAGACGAGCG-3') for WIND4-2.

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